

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: JEFFREY M. FRIEDMAN, YIYING ZHANG, RICARDO PROENCA
- (ii) TITLE OF INVENTION: OB POLYPEPTIDE ANTIBODIES AND METHOD OF MAKING
(AS AMENDED)
- (iii) NUMBER OF SEQUENCES: 21
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Klauber & Jackson
 - (B) STREET: 411 Hackensack Avenue
 - (C) CITY: Hackensack
 - (D) STATE: New Jersey
 - (E) COUNTRY: USA
 - (F) ZIP: 07601
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/204,730
 - (B) FILING DATE: December 3, 1998
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/292,345
 - (B) FILING DATE: August 17, 1994
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Jackson Esq., David A.
 - (B) REGISTRATION NUMBER: 26,742
 - (C) REFERENCE/DOCKET NUMBER: 600-1-087DIV
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 201 487-5800
 - (B) TELEFAX: 201 343-1684
 - (C) TELEX: 133521

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 701 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
 - (A) DESCRIPTION: Murine ob cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

Ile Ala Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala
100 105 110

Phe Ser Lys Ser Cys Ser Leu Pro Gln Thr Ser Gly Leu Gln Lys Pro
115 120 125

Glu Ser Leu Asp Gly Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val
130 135 140

Val Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln
145 150 155 160

Leu Asp Val Ser Pro Glu Cys
165

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 700 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
 - (A) DESCRIPTION: Human ob cDNA where N represents any nucleotide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Human
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 46..546
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

NNNGNNGTTG CAAGGCCCAA GAAGCCCAANN NTCCTGGGAA GGAAA ATG CAT TGG	54
Met His Trp	
1	
GGA ACC CTG TGC GGA TTC TTG TGG CTT TGG CCC TAT CTT TTC TAT GTC	102
Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr Leu Phe Tyr Val	
5 10 15	
CAA GCT GTG CCC ATC CAA AAA GTC CAA GAT GAC ACC AAA ACC CTC ATC	150
Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile	
20 25 30 35	
AAG ACA ATT GTC ACC AGG ATC AAT GAC ATT TCA CAC ACG CAG TCA GTC	198
Lys Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val	
40 45 50	
TCC TCC AAA CAG AAA GTC ACC GGT TTG GAC TTC ATT CCT GGG CTC CAC	246
Ser Ser Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His	
55 60 65	

CCC ATC CTG ACC TTA TCC AAG ATG GAC CAG ACA CTG GCA GTC TAC CAA Pro Ile Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln 70 75 80	294
CAG ATC CTC ACC AGT ATG CCT TCC AGA AAC GTG ATC CAA ATA TCC AAC Gln Ile Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn 85 90 95	342
GAC CTG GAG AAC CTC CGG GAT CTT CTT CAC GTG CTG GCC TTC TCT AAG Asp Leu Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys 100 105 110 115	390
AGC TGC CAC TTG CCC TGG GCC AGT GGC CTG GAG ACC TTG GAC AGC CTG Ser Cys His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu 120 125 130	438
GGG GGT GTC CTG GAA GCT TCA GGC TAC TCC ACA GAG GTG GTG GCC CTG Gly Gly Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu 135 140 145	486
AGC AGG CTG CAG GGG TCT CTG CAG GAC ATG CTG TGG CAG CTG GAC CTC Ser Arg Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu 150 155 160	534
AGC CCT GGG TGC TGAGGCCTT GAAGGTCCTT CTTCCTGCAA GGACTNACGT Ser Pro Gly Cys 165	585
TAAGGGAAGG AACTCTGGTT TCCAGGTATC TCCAGGATTG AAGAGCATTG CATGGACACC	645
CCTTATCCAG GACTCTGTCA ATTTCCCTGA CTCCTCTAAG CCACTCTTCC AAAGG	700

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 167 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (A) DESCRIPTION: Human ob polypeptide

(vi) ORIGINAL SOURCE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr Leu 1 5 10 15
Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys 20 25 30
Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr 35 40 45
Gln Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro 50 55 60
Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala 65 70 75 80

Val Tyr Gln Gln Ile Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln
85 90 95

Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala
100 105 110

Phe Ser Lys Ser Cys His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu
115 120 125

Asp Ser Leu Gly Gly Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val
130 135 140

Val Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln
145 150 155 160

Leu Asp Leu Ser Pro Gly Cys
165

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 166 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Murine ob polypeptide lacking Gln at position

49

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Cys Trp Arg Pro Leu Cys Arg Phe Leu Trp Leu Trp Ser Tyr Leu
1 5 10 15

Ser Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys
20 25 30

Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr
35 40 45

Ser Val Ser Ala Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly
50 55 60

Leu His Pro Ile Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Val
65 70 75 80

Tyr Gln Gln Val Leu Thr Ser Leu Pro Ser Gln Asn Val Leu Gln Ile
85 90 95

Ala Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Phe
100 105 110

Ser Lys Ser Cys Ser Leu Pro Gln Thr Ser Gly Leu Gln Lys Pro Glu
115 120 125

Ser Leu Asp Gly Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val
130 135 140

Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu
145 150 155 160

Asp Val Ser Pro Glu Cys
165

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 166 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (A) Description: Human ob polypeptide lacking Gln at position 49

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr Leu
1 5 10 15

Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys
20 25 30

Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr
35 40 45

Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly
50 55 60

Leu His Pro Ile Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val
65 70 75 80

Tyr Gln Gln Ile Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile
85 90 95

Ser Asn Asp Leu Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe
100 105 110

Ser Lys Ser Cys His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp
115 120 125

Ser Leu Gly Gly Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val
130 135 140

Ala Leu Ser Arg Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu
145 150 155 160

Asp Leu Ser Pro Gly Cys
165

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (primer)
 - (A) DESCRIPTION: PCR 5 primer for exon 2G7
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAGGGCAGG AAAATGTG

18

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (primer)
 - (A) DESCRIPTION: PCR 3 primer for exon 2G7
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATCCTGGAC TTTCTGGATA GG

22

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 176 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
 - (A) DESCRIPTION: exon 2G7
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGCAAGAAG AAGAAGATCC CAGGGCAGGA AAATGTGCTG GAGACCCCTG TGTCGGGTCC	60
NGTGGNTTTG GTCCTATCTG TCTTATGTNC AAGCAGTGCC TATCCAGAAA GTCCAGGATG	120
ACACCAAAAAG CCTCATCAAG ACCATTGTCA NCAGGATCAC TGANATTTC CACACG	176

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (A) DESCRIPTION: putative N-terminal signal sequence of Murine Ob Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Phe	leu	trp	leu	trp	ser	tyr	leu	ser	tyr	val	gln	ala	val	pro
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 287 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (plasmid)

- (A) DESCRIPTION: pET-15b expression vector

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: T7 promoter
- (B) LOCATION: 20..37

(ix) FEATURE:

- (A) NAME/KEY: lac operator
- (B) LOCATION: 39..64

(ix) FEATURE:

- (A) NAME/KEY: His-Tag
- (B) LOCATION: 123..137

(ix) FEATURE:

- (A) NAME/KEY: Thrombin cleavage site
- (B) LOCATION: 184..196

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AGATCTCGAT CCCGCGAAAT TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA      60
TTCCCCTCTA CAAATAATTT TGTTTAACTT TAAGAAGGAG ATATACC ATG GGC AGC      116
                                     1
AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC      164
      5              10              15
CAT ATG CTC GAG GAT CCC GCT GCT AAC AAA GCC CGA AAG GAA GCT GAG      212
      20              25              30              35
TTG GCT GCT GCC ACC GCT GAG CAA TAA CTA G CATAACCCCT TGGGGCCTCT      263
      40
AAACGGGTCT TGAGGGGTTT TTG      287
```

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (primer)
 - (A) DESCRIPTION: Murine 5 primer
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
CTTATGTTCA TATGGTGCCG ATCCAGAAAG TC      32
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(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (primer)
 - (A) DESCRIPTION: Murine 3 primer
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCCCTCTACA TATGTCTTGG GAGCCTGGTG GC

32

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(A) DESCRIPTION: Human 5 primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCTATGTCCA TATGGTGCCG ATCCAAAAAG TC

32

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (primer)

(A) DESCRIPTION: Human 3 primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTCCTTCCCA TATGGTACTC CTTGCAGGAA GA 32

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Splice acceptor site in ob

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(ix) FEATURE:
 (A) NAME/KEY: Splice acceptor site

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGCAGTCGGT A

11

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
 (A) DESCRIPTION: abnormal splice acceptor site in ob
(iii) HYPOTHETICAL: no
(iv) ANTISENSE: No
(ix) FEATURE:
 (A) NAME/KEY: Splice acceptor site (without Glutamine)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AG CAG TCG GTA

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
 (A) DESCRIPTION: ob peptide fragment
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Val	Pro	Ile	Gln	Lys	Val	Gln	Asp	Asp	Thr	Lys	Thr	Leu	Ile	Lys	Thr
1				5					10					15	

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide
(A) DESCRIPTION: ob peptide fragment

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu	His	Pro	Ile	Leu	Ser	Leu	Ser	Lys	Met	Asp	Gln	Thr	Leu	Ala
1			5					10					15	

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide
(A) DESCRIPTION: ob peptide fragment

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser	Lys	Ser	Cys	Ser	Leu	Pro	Gln	Thr	Ser	Gly	Leu	Gln	Lys	Pro	Glu
1			5					10					15		

Ser Leu Asp

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide
(A) DESCRIPTION: ob peptide fragment

(v) FRAGMENT TYPE: Carboxyl terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ser	Arg	Leu	Gln	Gly	Ser	Leu	Gln	Asp	Ile	Leu	Gln	Gln	Leu	Asp	Val
1			5					10					15		

Ser Pro Glu Cys
20